Novel Mutations in the Gene Encoding Secreted Lymphocyte Antigen-6/Urokinase-type Plasminogen Activator Receptor-related Protein-1 (SLURP-1) and Description of Five Ancestral Haplotypes in Patients with Mal de Meleda

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Mal de Meleda is a recessive, transgressive palmoplantar keratoderma for which we previously identified mutations in the gene encoding secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related protein-1 (SLURP-1). In this report we describe two new mutations: (i) a founder mutation, which changes a conserved cysteine residue to tyrosine (C99Y) in a large inbred Tunisian pedigree, and (ii) a signal sequence mutation (W15R), which was homozygous in a German family and heterozygous in a Scottish patient. Four ancestral haplotypes were observed in 69 patients from countries around the Mediterranean basin, and an additional haplotype was found in the German and Scottish patients. Key words: palmoplantar keratoderma/Mal de Meleda/ARS (component B) mutations/secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor related protein-1/ancestral haplotypes/SLURP-1. ***J Invest Dermatol 120:351–355, 2003***

Mal de Meleda (MdM, MIM 248300) is a transgressive palmoplantar keratoderma associated with perioral erythema, hyperhidrosis, brachydactyly, nail abnormalities, and lichenoid plaques. The progressive character of the lesions can lead to severe functional handicap with reduced mobility of hands and feet. We previously reported the identification of mutations in the ARS (component B) gene, which encodes a secreted Ly6/uPAR (lymphocyte antigen 6/urokinase-type plasminogen activator receptor) related protein-1 (SLURP-1; Fischer *et al*, 2001). This protein is a non-glycosyl phosphatidyl inositol-anchored member of the Ly6/uPAR superfamily, in which most members have been localized in a cluster on chromosome 8q24.3. This receptor (uPAR) is known to interact with integrin (Simon *et al*, 2000). The amino acid composition of SLURP-1 is closely related to that of cytotoxins, such as snake venom α-neurotoxins and cardiotoxins (Ploug and Ellis, 1994). SLURP-1 may function as a ligand for a receptor yet to be identified and may interfere with weak intercellular adhesion as has been shown for the mouse Ly6d and its ligand Ly6d-L (Apostolopoulos *et al*, 2000).

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Abbreviations: SLURP-1, secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor related protein-1; MdM, Mal de Meleda; GH1, growth hormone 1.

We studied 11 nuclear families from the Sfax region in Tunisia comprising 22 patients and 60 nonaffected family members (**Fig 1**), four Algerian families, including six patients and 14 nonaffected family members, one German family originally from the Sudetenland, including one patient, his parents and a sister, and an isolated case from Scotland, whose ancestors were farmers from Ayrshire for several generations. With the exception of the Scottish patient whose parents were unrelated, all the families are consanguineous.

The clinical data and pedigree information were recorded by five dermatologists (B.B., C.M., S.Ma., S.Mo., H.T.). Tunisian patients presented similar progressive clinical features. Onset in early infancy was characterized by erythema of the palms and soles, rapidly followed by a diffuse yellowish hyperkeratosis. Keratoderma of the dorsal surface of the distal phalanges progressively extended to the dorsa of the hands and feet, involving the wrists and the ankles in the majority of cases. In most patients, the transgressive keratoderma included the elbows, knees, and ulnar side of the forearms. Yellow keratoderma of the palms and soles was usually outlined by a red scaly border. Hyperconvexity of the nails and conical distal phalanges were constant features of the disease, mainly in the adult patients. In elderly patients, contractures of the hands and fingers and sclerodactyly were common features. Hyperhydrosis, pseudo-ainhum, and brachydactyly were seen in some patients. The German, Scottish, and Algerian patients also presented typical symptoms of Mal de Meleda, as described previously (Bouadjar *et al*, 2000). Photographs of the hands in five patients with typical MdM are shown in **Fig 2**.

Blood samples were collected from each participant family member after obtaining written informed consent. DNA

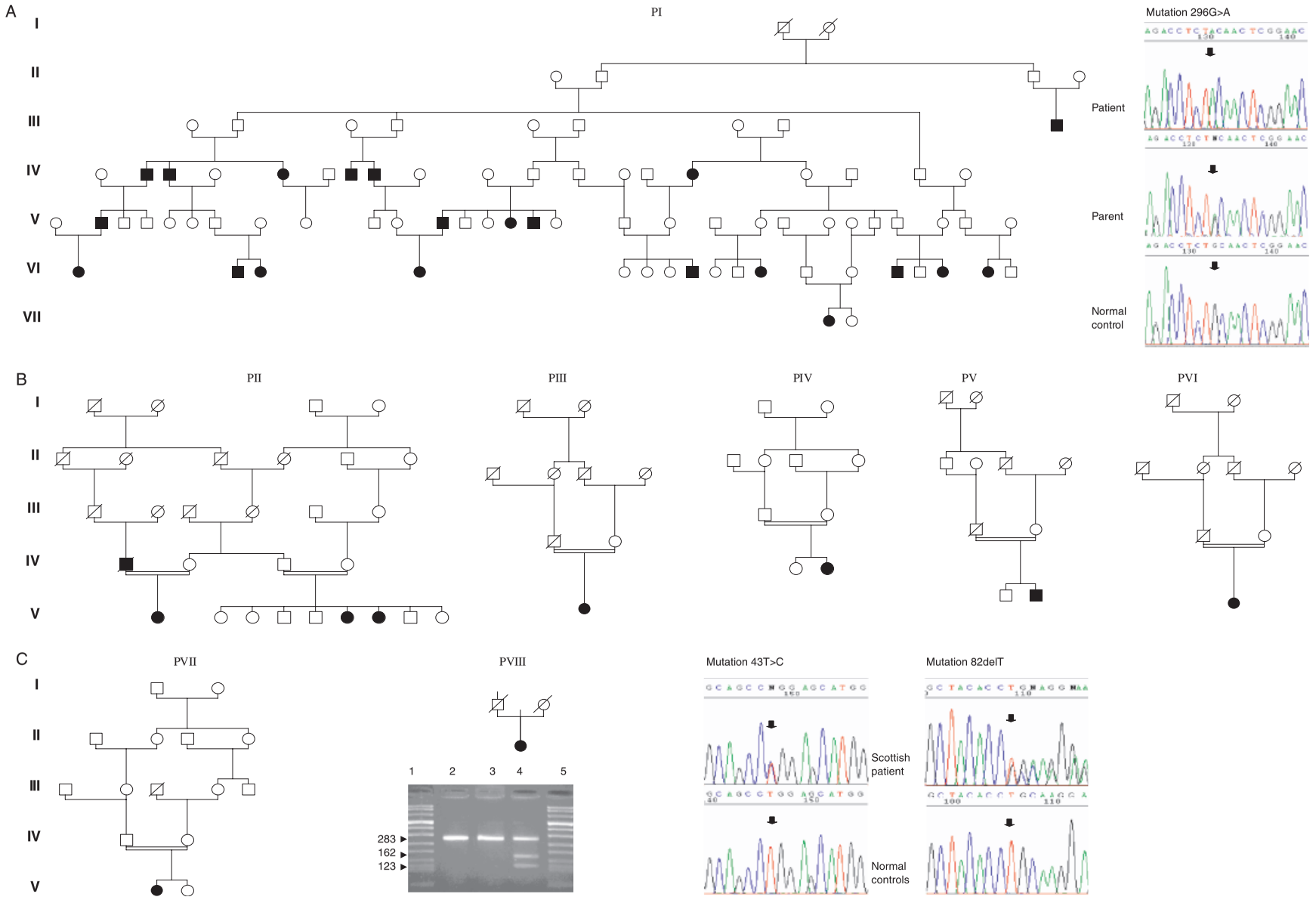

Figure 1. Pedigrees and mutations in the gene encoding SLURP-1 in MdM patients. Mutation sites are indicated by *arrows*. (*A*) Pedigree and DNA sequences from the large Tunisian kindred PI in which a G→A transition is present at nucleotide 296. (*B*) Other North African pedigrees reported in this study. PII, PIII, PIV, and PV are Algerian families, PVI is from Tunisia. PII, PIII, and PIV carry the 178 + 1g→a splice site mutation; PV and PVI have a deletion at nucleotide 82. (DNA sequences not shown.) (*C*) German (PVII) and Scottish (PVIII) pedigrees and DNA sequences. The Scottish patient is a compound heterozygote for the T→C transition at nucleotide 43 (which is present in the German family in the homozygous state) and a deletion at nucleotide 82. Restriction fragment analysis of the 43T→C mutation that creates a *Nci*I restriction site in exon 1. *Lanes 1 and 5*: Size marker VIII (Roche Molecular Biochemicals). *Lane 2*: Undigested PCR product of 283 bp. *Lane 3*: Enzymatic digestion of the PCR product of a normal control showing a single 283 bp band. *Lane 4*: Digestion of the exon 1 PCR product of the Scottish proband, who is heterozygous for the 43T→C mutation, showing 283 bp, 162 bp, and 121 bp bands.

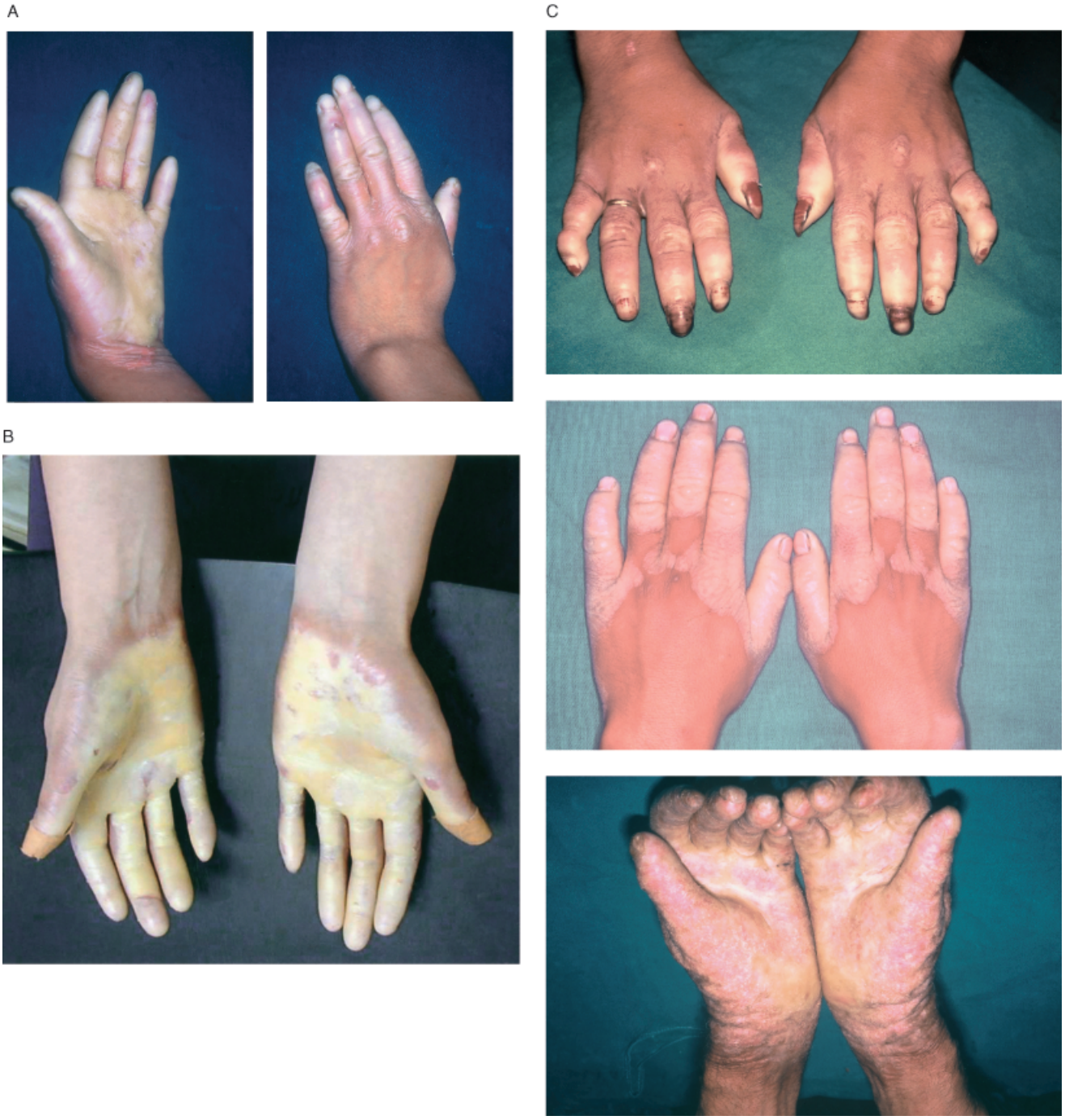

Figure 2. Typical aspect of palms and dorsal surfaces of the hands in five adult patients with Mal de Meleda. (*A*) The Algerian patient with a 178+1g>a splice site mutation shows yellow hyperkeratosis of palms, limited by an erythrodermic scaly border and whitish conical, sclerodermiform phalanges. Lesions are spread over dorsal surfaces in a glove-like distribution; thickening and clubbing of the nails with onycholysis (fifth finger) and conical phalanges. (*B*) Transgressive yellow hyperkeratosis of palms with red desquamative border in a German patient with the 43T→C mutation (W15R). (*C*) Three patients from the large Tunisian kindred with the 296G→A mutation (C99Y). *Top*: Brachydactyly, conical phalanges and pseudo-ainhum of the fifth finger in a female patient. Fingernails have been colored by the patient. *Middle*: Typical transgressive character of the lesions spread over the dorsal surfaces of the hand in the second patient. *Bottom*: Severe hyperkeratosis and contractures of the hands and the fingers in an elderly patient.

extraction from peripheral blood leukocytes was performed using standard procedures. Genotyping with 11 fluorescent markers was carried out in patients and nonaffected family members as described previously (Fischer *et al*, 2001). Linkage programs and parameters were also as previously described. Genotyping results showed linkage to 8q24, confirming genetic homogeneity of Mal de Meleda in the families we studied. The maximum pairwise LOD score for all families was 10.37 for the marker AFMa082wh9 (D8S1836) at $\theta = 0.00$, without incorporation of consanguineous loops unless patients were able to supply this information.

The central parts of the haplotypes of the patients in this study as well as those from a previous study (Fischer *et al*, 2001) are presented in **Table I**. Twenty-one of the 22 Tunisian patients presented the same homozygous haplotype (D) for nine of the 11 markers studied confirming the existence of consanguinity and a founder effect in this kindred, which included 10 nuclear

Table I. Five disease-associated haplotypes in 71 patients with Mal de Meleda

Haplotype	**A**	**B**	**C**	**D**	**E**
CNG003*	126	136	138	140	134
D8S1751*	155	149	149	149	149
D8S1836*	139	147	145	147	153
Exon1 (–60)**	C	C	C	G	C
Exon1 (–55)**	C	G	G	G	G
Mutation	82delT	178 + 1g > a	286C > T	296G > A	43T > C
Origin	Algeria/Croatia/Tunisia/Scotland	Algeria	Croatia	Tunisia	Germany/Scotland
No. of patients	32 (+1)	12	4	21	1 (+1)
No. of kindreds	15	6	3	1	2

The disease-associated haplotypes (A, B, C, D, and E) are contructed from the results of genotyping with microsatellite markers (CNG003, D8S1751, D8S1836) and SNPs** (single nucleotide polymorphisms) at the indicated positions 60 and 55 nucleotides upstream of the initiation codon of exon 1; the numbers represent the allele sizes of the amplimers in base pairs.* The Scottish patient, who was a compound heterozygote and therefore exhibited two different haplotypes (*A, E*), was counted twice (*in parentheses*). Patients and kindreds include data from Fischer *et al* (2001).

Table II. Mutations in the gene encoding SLURP-1 in Mal de Meleda

Mutation	Location	Effect	Patient origin	Reference
1A → C	Exon1 Initiation codon	No translation	United Arab Emirates	Eckl *et al* (2001)[2]
43T → C	Exon 1	W15R	Germany, Scotland	This report
82delT	Exon 2	Frameshift → stop codon at aa 32, truncated protein	Algeria, Croatia, Scotland, Tunisia	Fischer *et al* (2001) and this study
178 + 1 g → a	Intron 2	Aberrant splicing	Algeria	Fischer *et al* (2001) and this study
256G → A	Exon 3	G86R	Palestine	Eckl *et al* (2001)[2]
286C → T	Exon 3	R96X → truncated protein	Croatia	Fischer *et al* (2001)
296G → A	Exon 3	C99Y	Tunisia	This report

families. Common ancestors were later confirmed by re-interviewing the nuclear families whose members lived near the coastal town of Sfax. The haplotypes of the four Algerian families (A and B) and the other Tunisian family (A) were identical to ancestral haplotypes previously observed in families from Algeria and Croatia (**Table I**). The German patient was homozygous for a different haplotype (E), which was present in a heterozygous state in the Scottish patient. The other haplotype in the Scottish patient was the same as ancestral haplotype A. Haplotypes in the latter had to be deduced, because parental DNA was not available.

Sequencing of both strands of the three exons and exon/intron boundaries of the ARS (component B) gene was performed in all 17 families (including the isolated case from Scotland) for all affected patients and both parents, and in supplementary non-affected siblings in cases of missing parents. Intronic oligonucleotide primers were defined for exon 1 (forward: 5′-TACCACGTTCCTGACTCACA-3′; reverse: 5′-CTAAGGAGG-CTCTCAGCCA-3′), exon 2 (forward: 5′-CCTTGAAAGATGT-CAGCGAG-3′; reverse: 5′-GTGTGGCAGCCTGTTCTG-3′) and exon 3 (forward: 5′-AGTGAGGGTTCTGACACTGG-3′; reverse: 5′-CCAGGAAGGACAAAAGTCAT-5′). Polymerase chain reaction (PCR) conditions were as previously described (Fischer *et al*, 2001).

Mutation analysis revealed a new homozygous mutation in the patients from the large Tunisian kindred, 296 G → A, resulting in a change from cysteine to tyrosine at amino acid position 99 (C99Y) (**Fig 1**). This mutation disrupts a disulfide bridge that is highly conserved in the Ly-6/uPAR superfamily (Adermann *et al*, 1999). Three Algerian families shared a homozygous splice site mutation (178 + 1g → a), which leads to aberrant splicing. The eleventh Tunisian family and one Algerian family had a homozygous deletion (82delT), leading to a frameshift and creation of a premature stop codon at amino acid position 32. These latter two mutations have been reported previously (Fischer *et al*, 2001). The German and Scottish patients presented a new missense mutation (43 T → C), which changes tryptophan to arginine (W15R) in the signal sequence of the protein (**Fig 1*C***). For the Scottish patient, the novel heterozygous mutation 43T → C in exon 1 was confirmed by digesting the PCR product of exon 1 with the restriction enzyme *Nci*I (Sigma-Aldrich, Saint Quentin, Fallavier, France) according to the manufacturer's instructions (**Fig 1*C***). This mutation was homozygous in the German patient, but the Scottish patient was a compound heterozygote in which the second mutation was the previously reported deletion (82delT). The most likely cleavage site for the peptide signal sequence is either between amino acids 18 and 19 or between 23 and 24 depending on the prediction program used (PSORT, TargetP, ExPASy; Nielsen *et al*, 1997). The W15R mutation in which a hydrophobic amino acid is replaced by a hydrophilic one corresponds to the border of the core hydrophobic region, which is known to be important for secretion (Izard and Kendall, 1994; Izard *et al*, 1996). A total of 50 unaffected individuals (100 chromosomes) from North Africa and Europe were analyzed for the presence of the two novel mutations; no sequence variations were found at these positions. Mutations in peptide signal sequences have already been reported in autosomal dominant disorders (Repaske *et al*, 1997; Siggaard *et al*, 1999) and as homozygous mutations in autosomal recessive diseases (Seppen *et al*, 1996; Sunthornthepvarakul *et al*, 1999; Fujita *et al*, 2000), but it is unusual to find one mutation in a signal sequence and another in the secreted part of

the protein in a recessive disorder. Duquesnoy *et al* (1990)[1] described two siblings with growth hormone deficiency (IGHD IA, MIM 262400) who were found to be compound heterozygotes for a 6.7 kb deletion in the GH1 gene and a frameshift mutation in the signal peptide coding region, which prevented synthesis of GH1.

Combining results from a previous study with those reported here, we found mutations in the ARS (component B) gene in all 71 patients from 27 families with a confirmed diagnosis of MdM. There was very little phenotypic heterogeneity; with few exceptions all five mutations led to a similar clinical panorama when age differences were taken into consideration. The question of genetic heterogeneity remains unresolved, however; Eckl *et al* (2001)[2] and Lestringant *et al* (2001) have described families with MdM in which linkage to 8qter could not be demonstrated, and Van Steensel *et al*, 2002) reported a MdM patient in which no mutation in the ARS (component B) gene could be found.

Together with the three mutations already reported, and two other mutations that were described in an abstract,[2] this brings the total number of mutations reported in MdM to seven (**Table II**). Three of these are missense mutations (W15R, G86R, and C99Y), two lead to premature stop codons (82delT, which creates a stop codon at amino acid 32, and 286 C→T, which changes codon 96, which normally encodes arginine to a stop codon [R96X]); one affects a splice site (178 + 1g→a) and one changes A to C in the initiation codon of exon 1. Two of the missense mutations involve changing nonpolar to basic amino acids (W15R and G86R) and one (C99Y) disrupts a conserved disulfide bridge.

We also found two additional sequence variations, 55 (C→G) and 60 (C→G) nucleotides upstream of the ATG start codon in exon 1 (**Table I**). These neutral polymorphisms were not disease-associated, as they were present in both healthy and affected individuals; however in all five ancestral haplotypes, there is a complete association between the alleles of the three central microsatellite markers, the single nucleotide polymorphisms and the corresponding mutation. For instance, all patients with the homozygous 82delT mutation from the 13 previously reported and 2 newly analyzed families were homozygous for C at both of these polymorphic sites, despite their diverse geographic origins (Algeria, Tunisia, Croatia, including Meleda island, Scotland). This might represent a founder effect originating in Serbo-Croatia and spread by Serbo-Croatian sailors at the time of the Dubrovnik league (fourteenth to seventeenth centuries), when these seamen transported cargo throughout the Mediterranean and beyond under the patronage of the Turkish Empire (Goodwin, 1999; Bakija-Konsuo *et al*, 2002).

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ELECTRONIC DATABASE

GenBank and SwissProt accession numbers: ARS component B gene, X99977; SLURP-1, P55000.
ExPASy: http://www.expasy.ch/
PSORT: http://psort.nibb.ac.jp/
TargetP: http://www.cbs.dtu.dk/services/TargetP/

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